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(54) HUMAN SERUM ALBUMIN-TIMP2 FUSION PROTEIN, A POLYNUCLEOTIDE ENCODING THE SAME AND A METHOD OF PRODUCING THE HUMAN SERUM ALBUMIN-TIMP2 FUSION PROTEIN

(57) Abstract:

The present invention provides a human serum albumin-TIMP2 fusion protein having the amino acid sequence set forth in SEQ ID NO. 10, a polynucleotide encoding the same and a vector comprising the polynucleotide, a host cell transformed with the vector, a method for producing the human serum albumin-TIMP2 fusion protein and a pharmaceutical composition comprising the human serum albumin-TIMP2 fusion protein. The human serum albumin-TIMP2 fusion protein is stable and retains the activity of TIMP2, thus it can be used as a pharmaceutical composition to treat diseases related to angiogenesis and/or metastasis of cancer cells.

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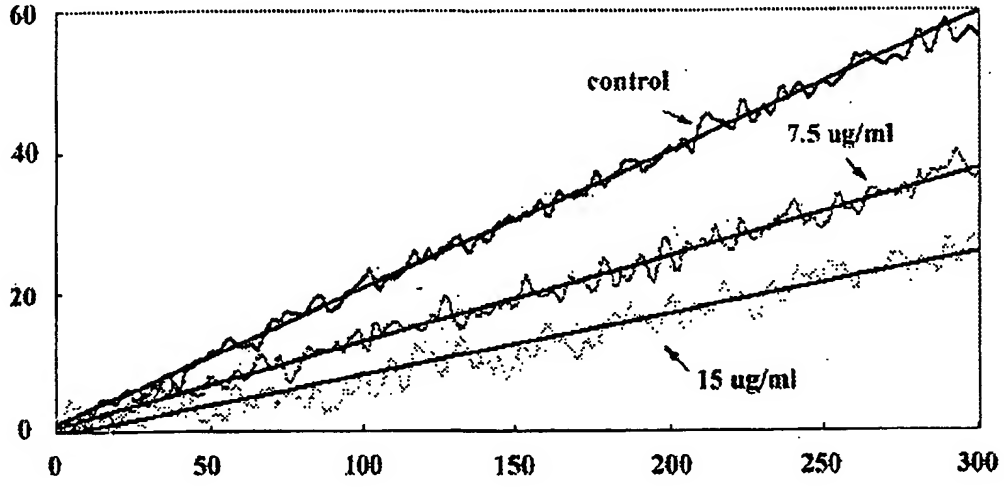
(54) Title: HUMAN SERUM ALBUMIN-TIMP2 FUSION PROTEIN, A POLYNUCLEOTIDE ENCODING THE SAME AND A METHOD OF PRODUCING THE HUMAN SERUM ALBUMIN-TIMP2 FUSION PROTEIN



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HUMAN SERUM ALBUMIN-TIMP2 FUSION PROTEIN, A POLYNUCLEOTIDE ENCODING THE SAME AND A METHOD OF PRODUCING THE HUMAN SERUM ALBUMIN-TIMP2 FUSION PROTEIN

Technical Field

The present invention relates to a fusion protein, and particularly, to a human serum albumin-TIMP2 fusion protein, a polynucleotide encoding the same, a vector comprising the polynucleotide, a transformed host cell comprising the vector, a pharmaceutical composition comprising the human serum albumin-TIMP2 fusion protein and a method for producing the human serum albumin-TIMP2 fusion protein.

Background Art

Angiogenesis is the process of generating new capillary blood vessels. During angiogenesis, neovascularization is tightly regulated and activation thereof occurs in embryogenic development, tissue remodeling, wound healing, and periodic cycles of corpus luteum development (Folkman and Cotran, *Int. Rev. Exp. Pathol.*, **16**, 207-248, 1976).

During the process of angiogenesis, capillary blood vessel endothelial cells start to proliferate from an existing vasculature. The endothelial cells grow very slowly as compared with other types of cells in a body. The proliferation of these cells is induced by pro-angiogenic cytokines, inflammation mediators, and activated proteolytic enzymes.

Failure to regulate angiogenesis leads to the development of several clinical syndromes or conditions. Pathological angiogenesis is involved in various diseases such as cancer in metastatic phase, arthritis, psoriasis, and retinopathy.

Not only reorganization of the blood vessel by migration, proliferation and differentiation of endothelial cells, but also degradation of an extracellular matrix is required for angiogenesis. One of the major events for inducing angiogenesis is a breakdown of the extracellular matrix before the formation of the capillary blood vessels. One of the most important enzymes which are involved in the matrix degradation is matrix metalloproteinase (MMP), a family of over 20 proteins. MMPs are endopeptidases, which degrade or proteolyze various components of the extracellular matrix such as collagen, proteoglycan, and gelatin.

An MMP's activity is modulated by an endogenous substance called Tissue Inhibitors of Metalloproteinases (TIMP) (Liotta and Stetler-Stevenson, *Semin. Cancer. Biol.* 1(2), 99-106, 1990; Liotta *et al.*, *Cell*, 64(2), 327-336, 1991). The proteins in the TIMP family are classified as tumor suppressor proteins and four proteins have been identified as members of this family.

TIMP2, one of the four identified proteins in TIMP family, is able to bind to pro- and active form of MMP-2. Since TIMP2 inhibits all the activated forms of MMPs, TIMP2 acts as a key inhibitor molecule in angiogenesis and cancer metastasis. For example, tumor cell growth and metastasis were inhibited by a gene therapy with TIMP2 in experimental animals (Hajitou *et al.*, *Cancer Res.*, 61, 3450-3457, 2001; Li *et al.*, *Human Gene Ther.* 12, 515-526, 2001; Sacco *et al.*, *Gene Ther.*, 8, 67-70, 2001). However, studies with TIMP2 were very limited due to a very limited amount of the protein existing in a biological system. Therefore, it is indispensable to develop a recombinant technique for overexpressing the TIMP-2 protein *in vitro*.

Although *E. coli* is a preferred host in recombinant DNA technology for producing large quantities of heterologous proteins economically, certain foreign proteins expressed in large quantities from *E. coli* are precipitated as inclusion bodies. Recovery of a biologically active protein from these inclusion bodies has presented critical problems and the recovered proteins are often biologically inactive because they are folded into a three-dimensional conformation different from that of native protein. Since TIMP2 has 6-disulfide linkages, it is very complicated to refold denatured TIMP2 into its correct, biologically active conformation.

As a eukaryote, yeast is a suitable host organism for a high-level production of secreted soluble cytosolic proteins of human origin. Indeed, many kinds of pharmaceutically important proteins have been expressed in yeast. Yeast is able to splice out introns and transport proteins through secretory pathways as higher eukaryotes do. Especially, *Saccharomyces cerevisiae*, the molecular and cellular biology of which has been intensively studied, has been exploited as a host for heterologous protein production since essential elements for gene expression such as strong and regulable promoters, vectors, and genetic markers are well developed (Romanos *et al.*, *Yeast*, 8, 423-488, 1992). Moreover, its use in food fermentation for thousands of years proved that *S. cerevisiae* causes no harm to human beings and the processes for the production of therapeutic proteins using yeast acquired GRAS (generally recognized as safe) status. Altogether, these features make *S. cerevisiae* one of the most suitable organisms for heterologous gene expression.

Despite many advantages of yeast expression systems, a number of proteins are neither expressed in a large quantity nor secreted efficiently in yeast for unknown reasons. When human TIMP2 is expressed in yeast, for example in *S. cerevisiae*, the expression level is extremely low.

Human serum albumin (HSA) consisting of 585 amino acids is the most abundant protein in plasma, representing about 60 % of total plasma proteins. A major function of serum albumin is to maintain a natural osmotic pressure of plasma and to transport sparingly soluble substances throughout the body. Serum albumin also functions as a carrier of endogenous and exogenous molecules, and for many years it has been thought to be devoid of any enzymatic function. However, recently, it has been found that it acts as dihydrotestosterone enolase and phospholipid cysteine peroxidase (Drmanovic *et al.*, *Anticancer Res.* 19(5B), 4113-4124, 1999; Hurst *et al.*, *Biochem J.*, 338(Pt3), 723-728, 1999). Despite these findings, exogenously administered modified serum albumins, for example recombinant therapeutic proteins fused to serum albumin, are not likely to contribute significantly to the total albumin pool because of the relative abundance of albumin in plasma. Furthermore, human serum albumin is a very stable protein displaying an *in vivo* half-life of 19 days in the adult human (Sterling, K., *J. Clin. Invest.*, 30, 1228, 1957).

Disclosure of the Invention

It is an object of the present invention to provide a human serum albumin-TIMP2 fusion protein.

It is another object of the present invention to provide a polynucleotide encoding the human serum albumin-TIMP2 fusion protein.

It is another object of the present invention to provide a vector comprising a polynucleotide encoding a human serum albumin-TIMP2 fusion protein.

It is another object of the present invention to provide a transformed host cell with a vector comprising a polynucleotide encoding the human serum

albumin-TIMP2 fusion protein.

It is another object of the present invention to provide a method for producing a human serum albumin-TIMP2 fusion protein.

It is yet another object of the present invention to provide a pharmaceutical composition comprising a human serum albumin-TIMP2 fusion protein.

The present invention provides a human serum albumin-TIMP2 fusion protein having the amino acid sequence of SEQ ID NO. 10. The fusion protein has an activity of inhibiting the MMP enzyme activity and angiogenesis. The molecular weight of the fusion protein is about 87.6 kDa. The fusion protein is made by fusing the carboxyl terminus of a human serum albumin to the amino terminus of TIMP2. When the fusion protein is linked to secretory signal sequence, it can be secreted to a medium more efficiently than the TIMP2. Moreover, the fusion protein is more stable than TIMP2 by being fused to a human serum albumin.

The present invention also provides a polynucleotide encoding a human serum albumin-TIMP2 fusion protein having the amino acid sequence of SEQ ID NO. 10. Preferably, the polynucleotide is a polynucleotide having nucleotide sequence set forth in SEQ ID NO. 3.

The present invention also provides a vector comprising a polynucleotide encoding a human serum albumin-TIMP2 fusion protein having the amino acid sequence of SEQ ID NO. 10. Preferably, the polynucleotide is a polynucleotide having nucleotide sequence set forth in SEQ ID NO. 3. The vector may include any element to establish a conventional function as a vector, for example, promoter, terminator, selection marker, and origin of replication. The promoter can be constitutive or regulative, and is selected from, for example, promoters of genes for galactokinase (GAL1), uridylyltransferase (GAL7), epimerase (GAL10), phosphoglycerate kinase (PGK), glyceraldehyde-3-phosphate dehydrogenase (GPD), alcohol dehydrogenase

(ADH), and the like. The vector may further include a polynucleotide encoding a secretory signal sequence to secrete the fusion protein into a medium. The polynucleotide includes a polynucleotide encoding a secretory signal sequence which is, for example, a polynucleotide encoding a human serum albumin presequence having the nucleotide sequence set forth in SEQ ID. 2. Generally, the secretory signal sequence guides heterologous proteins through the secretory pathways of a host cell, for example, a yeast cell and finally to the culture medium. Preferably, the vector is a pHSATIMP. Table 1 describes the components of pHSATIMP. FIG. 1 is a schematic representation of the pHSATIMP plasmid.

[Table 1]

	pHSATIMP
Promoter	*GAL10* promoter (SEQ ID NO. 1)
Signal sequence	HSA signal sequence (SEQ ID NO. 2)
Structural gene	HSA-TIMP2 fusion gene (SEQ ID NO. 3)
Terminator	*GAL7* terminator (SEQ ID NO. 4)
Selection marker	*URA3* gene
Origin of replication	2 μ *m*

The present invention also provides a host cell transformed with a vector comprising a polynucleotide encoding a human serum albumin-TIMP2 fusion protein having the amino acid sequence of SEQ ID NO. 10. Preferably, the polynucleotide is a polynucleotide having nucleotide sequence set forth in SEQ ID NO. 3. The host cell for the expression of the said polynucleotide, SEQ ID NO. 3, can be any cell, including yeasts, that can be used for the heterologous gene expression. As regards yeasts, preferred genera are *Saccharomyces, Pichia, Hansenula, Yarrowia, Kluyveromyces,* and *Schizosaccharomyces.* One example of the transformed host cell is *S. cerevisiae* JY28 [a strain (*MAT pep4::HIS3 prb-1.6R can1 his3-20 ura3-52*) carrying pHSATIMP] (KCTC

10131BP).

The present invention also provides a method for producing a human serum albumin-TIMP2 fusion protein by cultivating a host cell transformed with a vector comprising a polynucleotide encoding a human serum albumin-TIMP2 fusion protein having the amino acid sequence of SEQ ID NO. 10 in a suitable medium to produce the fusion protein and recovering the fusion protein. Preferably, the polynucleotide is a polynucleotide having nucleotide sequence set forth in SEQ ID NO. 3. One example of the transformed host cell is *S. cerevisiae* JY28 [a strain (*MAT pep4::HIS3 prb-1.6R can1 his3-20 ura3-52*) carrying pHSATIMP] (KCTC 10131BP). The medium varies depending on a selected host cell, and includes a conventional medium used for cultivating the selected host cell. For example, if the host cell is *S. cerevisiae* JY28 [a strain (*MAT pep4::HIS3 prb-1.6R can1 his3-20 ura3-52*) carrying pHSATIMP] (KCTC 10131BP), a minimal medium containing 6.7 grams of yeast nitrogen base (without amino acids) (YNB) (Difco), 20 grams of glucose, and 20 grams of agar per liter can be used for the maintenance. The transformed host cell can be induced to produce the human HSA-TIMP2 by cultivating for 2 days at 30 °C in an induction medium composed of 10 grams of yeast extract (Difco), 20 grams of Bacto-peptone (Difco), 10 grams of glucose, and 20 grams of galactose per liter. The host cell transformed with the vector containing the polynucleotide, SEQ ID NO. 3, expresses and secretes the recombinant fusion protein of about 87.6 kDa in size. The recombinant fusion protein defines human TIMP2 protein fused with human serum albumin, retaining the biological activity of human TIMP2 protein.

Conventional separation and purification methods for protein can be used to purify the human serum albumin-TIMP2 fusion protein of the present invention. For example, a salting out, a dialysis, an ion chromatography and an affinity chromatography can be used. When *S. cerevisiae* JY28 [a strain (*MAT pep4::HIS3 prb-1.6R can1 his3-20 ura3-52*) carrying pHSATIMP] (KCTC 10131BP) was used, the final concentration of the fusion protein in culture

supernatant was about 30-50 mg per liter in a flask culture. Yeast strains including *S. cerevisiae* are known to secrete only a little amount of TIMP2, but the secretion efficiency was increased more than 50- to 100-folds by fusing TIMP2 with human serum albumin protein in the present invention.

The present invention also provides a pharmaceutical composition comprising a pharmaceutically effective amount of the human serum albumin-TIMP2 fusion protein and a pharmaceutically acceptable diluent or carrier. The diluent or carrier can be any material conventionally used for a pharmaceutical composition comprising a protein. The recombinant fusion protein in the present invention not only inhibits the activity of MMPs (Figure 4) but also suppresses the tube formation of human vein umbilical cells (Figure 5). Therefore, the human serum albumin-TIMP2 fusion protein of the present invention retains biological activity of the TIMP2 protein, and the fusion protein is expected to be pharmaceutically useful without any undesirable side effects because serum albumin used as a fusion partner is known to be the most abundant protein in plasma. Thus, the fusion protein can be used as an anti-angiogenic protein. In particular, the fusion protein is a potent therapeutic agent to treat diseases related to angiogenesis and/or metastasis of cancer cells and may be more useful than TIMP2 itself because of its prolonged *in vivo* stability endowed by its fusion partner, human serum albumin.

Brief Description of the Drawings

FIG. 1 is a schematic representation of the pHSATIMP plasmid.

FIG. 2 shows the SDS-PAGE and Western blotting results of the culture supernatants of *S. cerevisiae* JY28 (Y2805/pHSATIMP).

FIG. 3 shows the SDS-PAGE for a purified recombinant HSA-TIMP2 fusion protein.

FIG. 4 shows the inhibitory activity of the purified recombinant

HSA-TIMP2 protein on MMP-2.

FIG. 5 shows the effect of the recombinant HSA-TIMP2 on the tube formation of human umbilical vein endothelial cells (HUVECs).

Best mode for carrying out the Invention

The following examples are intended to further illustrate the present invention. However, these examples are presented only for a better understanding of the present invention without limiting its scope.

Example 1: Construction of the recombinant expression vector

In the present example, pHSATIMP containing *GAL10* promoter, HSA signal sequence, HSA structural gene, TIMP2 structural gene, and *GAL7* terminator was prepared. The gene for human serum albumin was amplified by PCR. The template was the HSA gene in the plasmid pHSA (LeadBio, Inc) and the primers used were SEQ ID NO. 5 (forward primer with the recognition sequence for *Eco*RI) and SEQ ID NO. 6 (backward primer containing 15mers that are complementary to the primer of SEQ ID NO. 7). The gene for human TIMP2 was also amplified by PCR. The template was the TIMP2 gene in the plasmid pMY2 (AngioLab, Inc) and the primers used were SEQ ID NO. 7 (forward primer) and SEQ ID NO. 8 (backward primer with the recognition sequence for *Hind*III). The 15mers of 3' terminal sequence of the amplified

human serum albumin gene and the 15mers of 5' terminal sequence of the TIMP2 gene are complementary to each other. Thus, in-frame fusion of the human serum albumin and TIMP2 genes could be made by PCR using SEQ ID NO. 5 and SEQ ID NO. 8 as primers. The PCR product treated with restriction enzymes *Eco*RI and *Hind*III were ligated with the vector pHSA cut with *Eco*RI and *Hind*III, resulting in the recombinant vector, pHSATIMP.

Example 2: Construction of transformant

The plasmid, pHSATIMP prepared in Example 1 was introduced into *S. cerevisiae* Y2805 (*MAT pep4::HIS3 prb-1.6R can1 his3-20 ura3-52*) by lithium acetate method (Ito et al., *J. Bacteriol.* 153, 163-168, 1983). The selected transformants were further tested by further growing them on a synthetic complete medium without uracil. The finally selected transformant was named *S. cerevisiae* JY28 (Y2805/pHSATIMP) and deposited in KCTC (Korean Collection for Type Cultures) 10131BP on December 3, 2001.

Example 3: Expression of the human serum albumin and TIMP2 fusion protein

The transformant obtained in Example 2 was grown for 2 days at 30°C in 50 ml of YPDG medium (1% Yeast extract, 2% Proteose-peptone, 1% glucose, 2% galactose). When glucose in the medium was depleted, *GAL10* promoter was turned on by galactose and the HSA-TIMP2 fusion protein was

expressed and secreted. Culture supernatants (20 μl) taken after 24 and 48 hours were analyzed on an SDS-PAGE gel by staining the gel with coomassie blue or after immunoblotting using a rabbit polyclonal serum directed against HSA. Figure 2 displays the SDS-PAGE and Western blotting results of the culture supernatants of *S. cerevisiae* JY28 (Y2805/pHSATIMP). Fig. 2A is a result of SDS-PAGE of the culture supernatants with Coomassie blue staining. Fig. 2B is a result of Western blotting with a rabbit polyclonal serum directed to a human serum albumin. Lanes 1, 2, 3, and 4 indicate a molecular weight marker, a supernatant(10 μl) of a control strain (Y2805) culture after 48 hours of growth; a supernatant(10 μl) of a JY28 (Y2805/pHSATIMP) culture after 24 hours of growth, and a supernatant(10 μl) of a JY28 (Y2805/pHSATIMP) culture after 48 hours of growth, respectively. The HSA-TIMP2 fusion protein having the size of 87.6 kDa is clearly shown in lanes 3 and 4.

Example 4: Purification of the recombinant HSA-TIMP2 protein

In order to purify the HSA-TIMP2, yeast culture media of Example 3 was recovered after centrifugation at 10,000 x g for 10 min. Proteins in the supernatant were precipitated with 70% of ammonium sulfate solution. Pellets were collected by centrifugation for 30 min at 15,000 x g, and redissolved in 50 mM HEPES buffer (pH 8.0). After removal of ammonium sulfate by dialysis, the concentrated protein solution was subjected to DEAE-sepharose

(Pharmacia) column chromatography. The column was washed with 50 mM HEPES buffer, pH 8.0, and bound proteins were eluted with linear gradient of 0.1-0.5 M NaCl solution. HSA-TIMP2 was eluted at 0.24 M of NaCl, the protein was analyzed on SDS-PAGE. As shown in Figure 3, the molecular size of the purified recombinant HSA-TIMP2 fusion protein was 87.6 kDa. About 20 mg of the HSA-TIMP2 was obtained from 1L of culture media.

Example 5: Effect of the recombinant HSA-TIMP2 on Matrix Metalloproteinase activity

(1) Preparation of MMP

MMP-2 cDNA (GENEBANK No. XM_048244) was cloned and prepared from insect cells (Sf21 insect cell) by using a Baculovirus system.

The obtained MMP-2 cDNA was cloned to a pBlueBac4.5 transfer vector (Invitrogen, Cat no. V1995-20), and then transfected to Sf21 cells with a Bac-N-Blue Transfection Kit (Invitrogen, Cat no. K855-01). Sf21 cells were cultured in TNM-FH media (Sigma, St. Louis, MO, U.S.A) containing 10% fetal bovine serum at 27℃, then harvested and re-suspended at a concentration of 10^7 cell/㎖. The cell suspension was incubated with a virus containing the cloned gene for 1 hr at room temperature. Infected Sf21 cells were grown for 72 hrs and the medium was recovered, and the MMP-2 was purified using a gelatin-sepharose affinity column (Sigma, G5384) chromatography.

(2) Inhibition of MMP activity

In order to investigate MMP inhibition by the recombinant HSA-TIMP2 fusion protein, MMP activity was assayed by a spectrofluorometric method using Perkin-Elmer LS50B.

Purified MMP-2 was used after activation with 1 mM APMA before assay. The substrate for MMP-2 was MCA-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2 (Bachem, Cat. No. M-1895).

For control, 2 ㎖ of reaction buffer (50 mM Tricine, pH 7.5, 10 mM $CaCl_2$, 200 mM NaCl) comprising DMSO, 10 nM of MMP-2 and 10 μM of substrate was prepared in a cuvette, and fluorescence intensity was measured for 5-10 min. at room temperature with a spectrofluorometer under an excitation wavelength of 328 nm and an emission wavelength of 393 nm.

HSA-TIMP2 was added to a reaction buffer containing a substrate and MMP-2, and fluorescence intensity was measured in the same manner.

Figure 4 shows the inhibitory activity of the purified recombinant HSA-TIMP2 fusion protein on MMP-2. As shown in Figure 4, 7.5 μg/ml and 15 μg/ml of HSA-TIMP2 inhibited about 37% and 55% of MMP-2 activity, respectively.

Example 6: Effect of the recombinant HSA-TIMP2 fusion protein on tube formation of HUVEC

The effect of the recombinant HSA-TIMP2 fusion protein on human

endothelial cells was investigated to evaluate the biological effect of the HSA-TIMP2 of the present invention. Since MMPs are responsible for the degradation of extracellular matrix, TIMP2 is able to inhibit the formation of tubular network of vessel, which represents migration and differentiation of endothelial cell.

Blood vessel endothelial cells, human umbilical vein endothelial cells (HUVECs), were isolated from freshly obtained cords after a cesarean section according to Grants' method (Grants *et al., Cell*, 58, 933-943, 1989). They were identified by immunocytochemical staining with anti-Factor VIII antibody. HUVECs cultured on Matrigel (BD Bioscience, Bedford, MA, USA) were treated with 6.5 μg/ml of HSA-TIMP2, and further incubated at 37℃ for 8-16 hrs. For control, the procedure was repeated with a solution without the recombinant HAS-TIMP2 fusion protein.

Fig. 5 shows the effect of the recombinant HAS-TIMP2 on a tube formation of human umbilical vein endothelial cells (HUVECs). Fig. 5A shows that a tubular network was formed as a process of neovascularization, when the HUVECs were grown on Matrigel. However, the microvascular network was disconnected when the HUVECs grown on Matrigel were treated with 6.5 μg/ml of HSA-TIMP2 (Fig. 5B). These data show that HSA-TIMP2 is able to inhibit angiogenesis by inhibiting MMP activity.

When the area of the tubular network of HUVECs was determined using an image analysis program, Image-Pro Plus® (Media Cybernetics, USA), the tube area after treatment of HSA-TIMP2 was about 19% as compared with the untreated control. That is, the tube formation was inhibited by 81% with 6.5

μg/ml of HSA-TIMP2 of the present invention.

Industrial Applicability

According to the human serum albumin-TIMP2 fusion protein of the present invention, it retains the biological activity of the TIMP2 and can be used as a pharmaceutically active component without any undesirable side effects.

According to the polynucleotide and the vector comprising the same of the present invention, it is able to express the human serum albumin-TIMP2 fusion protein of the invention.

According to the pharmaceutical composition of the present invention, it can be used as an anti-angiogenic protein or as a potent therapeutic agent to treat diseases related to angiogenesis and/or metastasis of cancer cells.

According to the method of the present invention, the human serum albumin-TIMP2 fusion protein of the present invention can be produced on a large scale by using a transformed host cell.

Applicant's or agent's file reference	International application No.

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 6, line 22.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

Korean Collection for Type Cultures

Address of depositary institution *(including postal code and country)*

Korea Research Institute of Bioscience and Biotechnology(KRIBB)
#52, Oun-dong, Yusong-ku, Taejon 305-333 Rep. of Korea

Date of deposit	Accession Number
03 December 2001	KCTC 10131BP

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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What is claimed is:

1. A polypeptide having the amino acid sequence set forth in SEQ ID NO. 10.

2. A polynucleotide encoding the polypeptide according to claim 1.

3. The polynucleotide of claim 2, wherein the polynucleotide is a polynucleotide having the nucleotide sequence set forth in SEQ ID NO. 3.

4. A vector comprising a polynucleotide encoding the polypeptide having the amino acid sequence set forth in SEQ ID NO. 10.

5. The vector of claim 4, wherein the polynucleotide is a polynucleotide having the nucleotide sequence set forth in SEQ ID NO. 3.

6. The vector of claim 4, further comprising a polynucleotide encoding a secretory signal sequence for extracellular secretion of a protein.

7. The vector of claim 6, wherein the secretory signal sequence comprises a polynucleotide encoding a human serum albumin presequence

8. The vector of claim 4, wherein the vector is pHSATIMP.

9. A host cell transformed with a vector according to any one of claims 4 through 8.

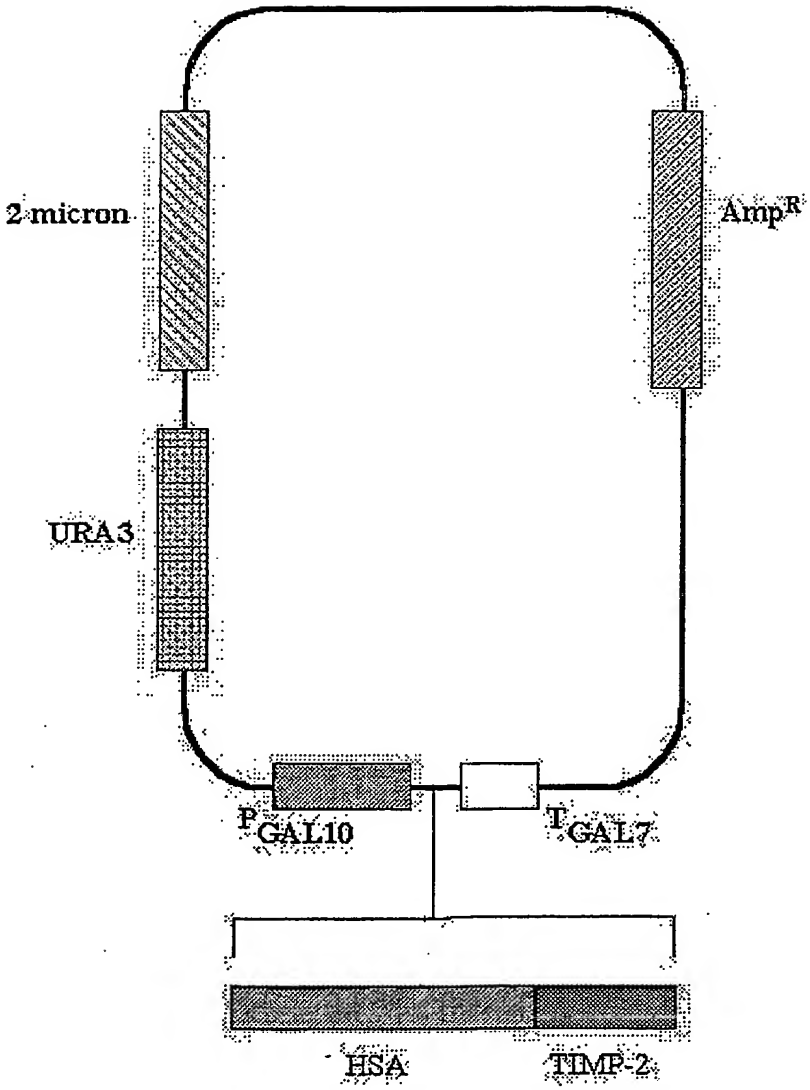
10. The host cell of claim 9, wherein the host cell is a yeast.

11. The host cell of claim 9, wherein the host cell is *S. cerevisiae* JY28 (KCTC 10131BP).

12. A method for producing a human serum albumin-TIMP2 fusion protein comprising cultivating the transformed host cell according to claim 9 in a suitable medium to produce the human serum albumin-TIMP2 fusion protein and recovering the human serum albumin-TIMP2 fusion protein.

13. A pharmaceutical composition comprising a pharmaceutically effective amount of a human serum albumin-TIMP2 fusion protein having the amino acid sequence set forth in SEQ ID. 10 and a pharmaceutically acceptable diluent or carrier.

Fig. 1



pHSATIMP

Fig. 2

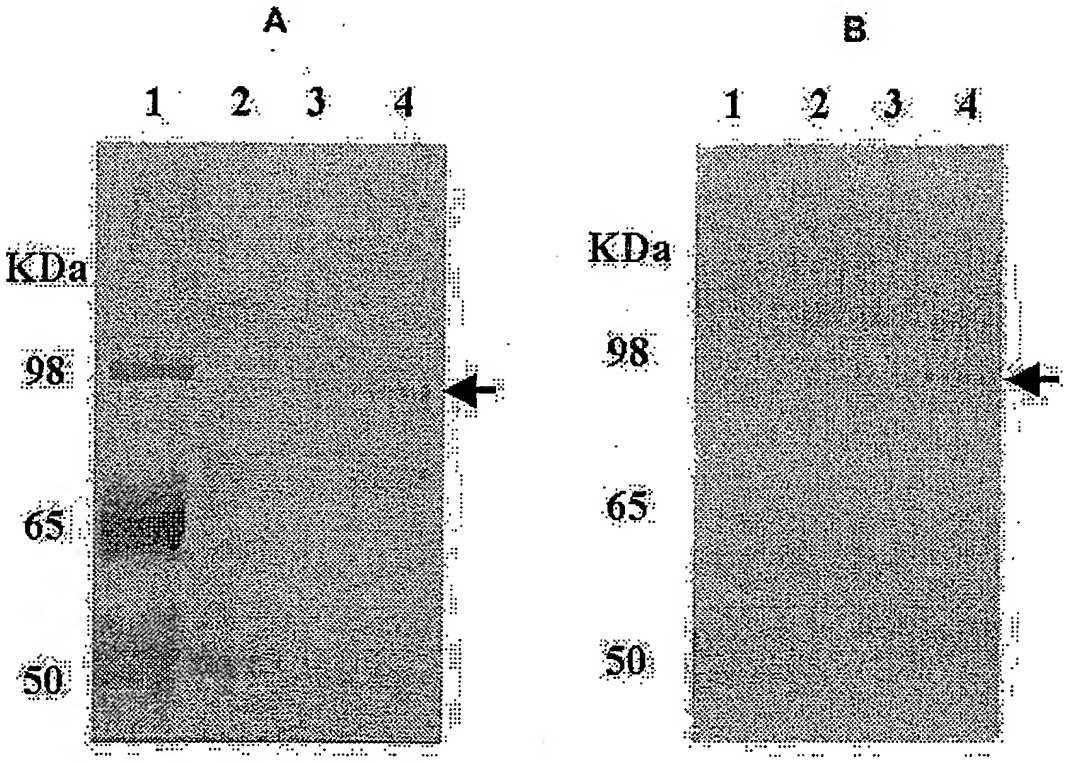


Fig. 3

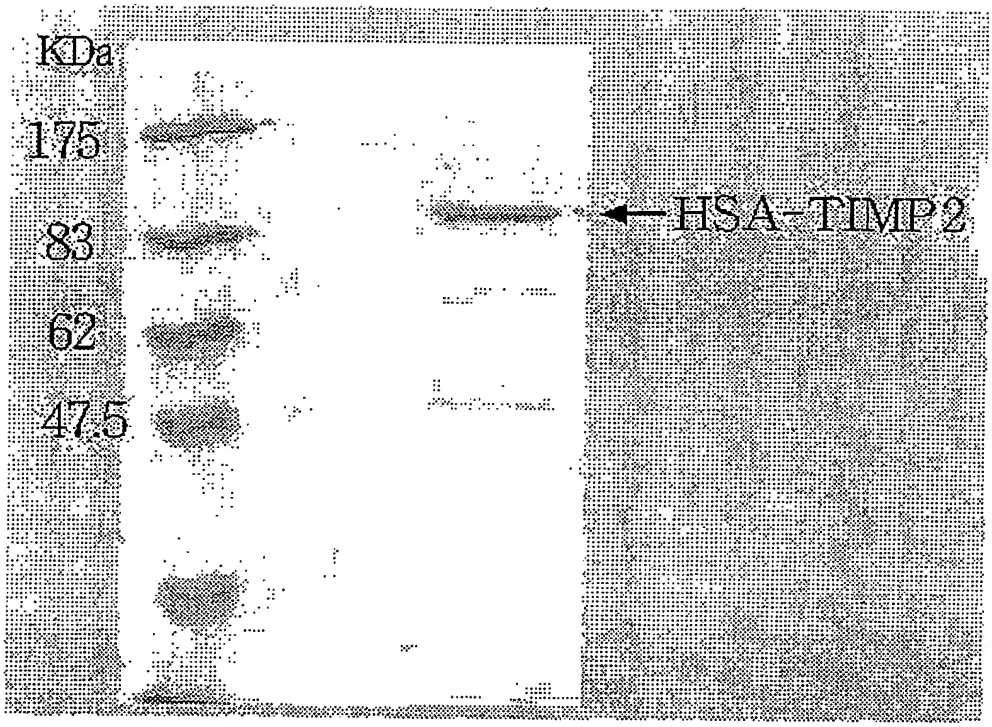


Fig. 4

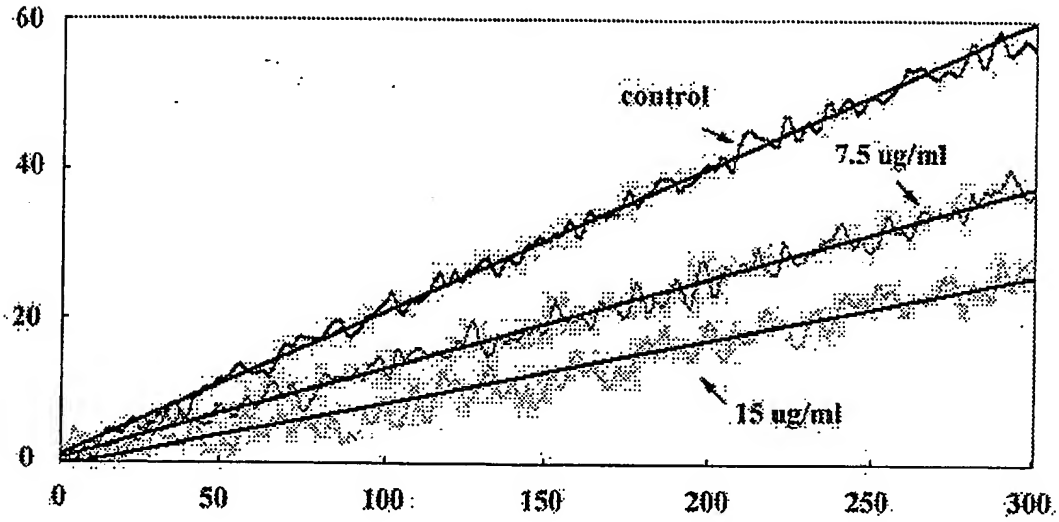


Fig. 5A

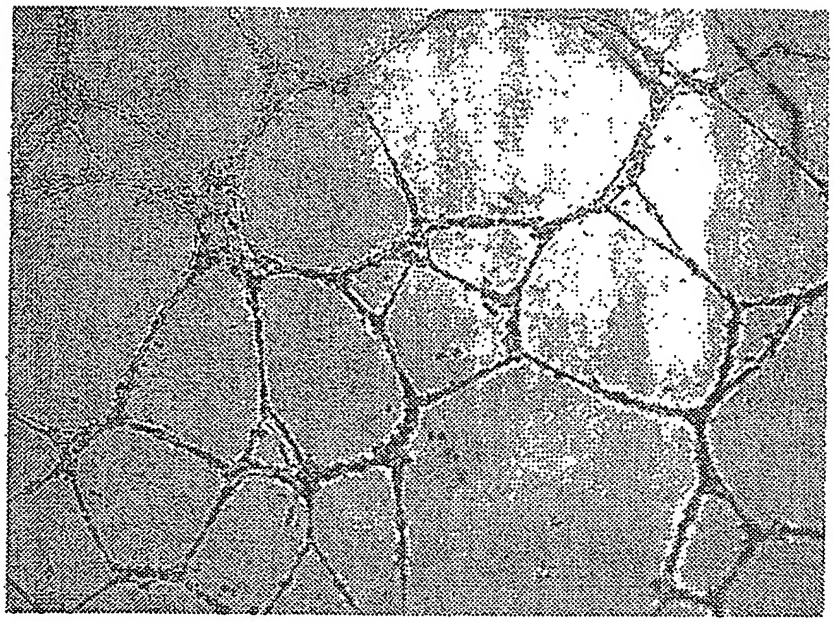
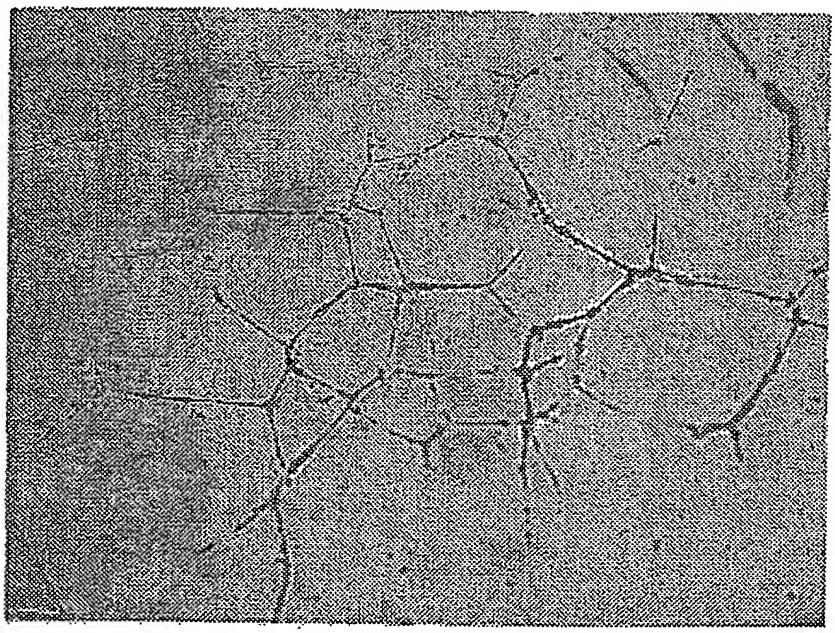


Fig. 5B



<110> ANGIOLAB, INC.
LeadBio.Inc.

<120> HUMAN SERUM ALBUMIN-TIMP2 FUSION PROTEIN, A POLNUCLEOTIDE ENCODING THE SAME AND A METHOD OF PRODUCING THE HUMAN SERUM
ALBUMIN-TIMP2 FUSION PROTEIN

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR03/00015

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12N 15/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01N 37/18 : C07K 14/475 : C12N 15/00, 15/11, 15/85, 15/63

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Patents and Applications for Inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubMed, Delphion, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Nomura N, Matsubara N, Horinouchi S, Beppu T. "Secretion by Saccharomyces cerevisiae of human apolipoprotein E as a fusion to serum albumin." Biosci Biotechnol Biochem., vol.59(3):532-4, March 1995 See the whole document	1-13
Y	Rajan SS, Lackland H, Stein S, Denhardt DT. "Presence of an N-terminal polyhistidine tag facilitates stable expression of an otherwise unstable N-terminal domain of mouse tissue inhibitor of metalloproteinase-1 in Escherichia coli." Protein Expr Purif., vol.13(1):67-72, June 1998 See the abstract	1-13
A	Cockett MI, Bebbington CR, Yarranton GT."High level expression of tissue inhibitor of metalloproteinases in Chinese hamster ovary cells using glutamine synthetase gene amplification." Biotechnology (NY), vol.8(7):662-7, July 1990 See the abstract	1-10
A	US 5,595,885A (The USA Department of Health and Human Services) Jan 1997 See the abstract	13
A	US 5,643,752A (Incyte Pharmaceuticals, Inc.) July 1997 See the abstract	1-12

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
26 MARCH 2003 (26.03.2003)	26 MARCH 2003 (26.03.2003)

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	AHN, Mi Jung Telephone No. 82-42-481-5593

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR03/00015

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5,595,885A	Jan 1997	None	
US 5,643,752A	July 1997	WO 9618725	June 1996